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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 5/02, 7/02, 7/50	A2	(11) International Publication Number: WO 92/138 (43) International Publication Date: 20 August 1992 (20.08.9

(21) International Application Number: PCT/US92/00916

(22) International Filing Date: 6 February 1992 (06.02.92)

(30) Priority data:
651,800 7 February 1991 (07.02.91) US

(60) Parent Application or Grant
(63) Related by Continuation
US 651,800 (CIP)
Filed on 7 February 1991 (07.02.91)

(71) Applicant (for all designated States except US): BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS [US/US]; 1737 West Polk, Suite 405, Chicago, IL 60612 (US).

(72) Inventor; and
(75) Inventor/Applicant (for US only) : KAHN, Michael [U. US]; 950 West Berwyn Street, Unit 12, Chicago, 60640 (US).

(74) Agent: HUGHES, A., Blair; Allegretti & Witcoff, Lt Ten South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), G (European patent), IT (European patent), JP, KR, L (European patent), MC (European patent), NL (European patent), SE (European patent), US.

Published
Without international search report and to be republish upon receipt of that report.

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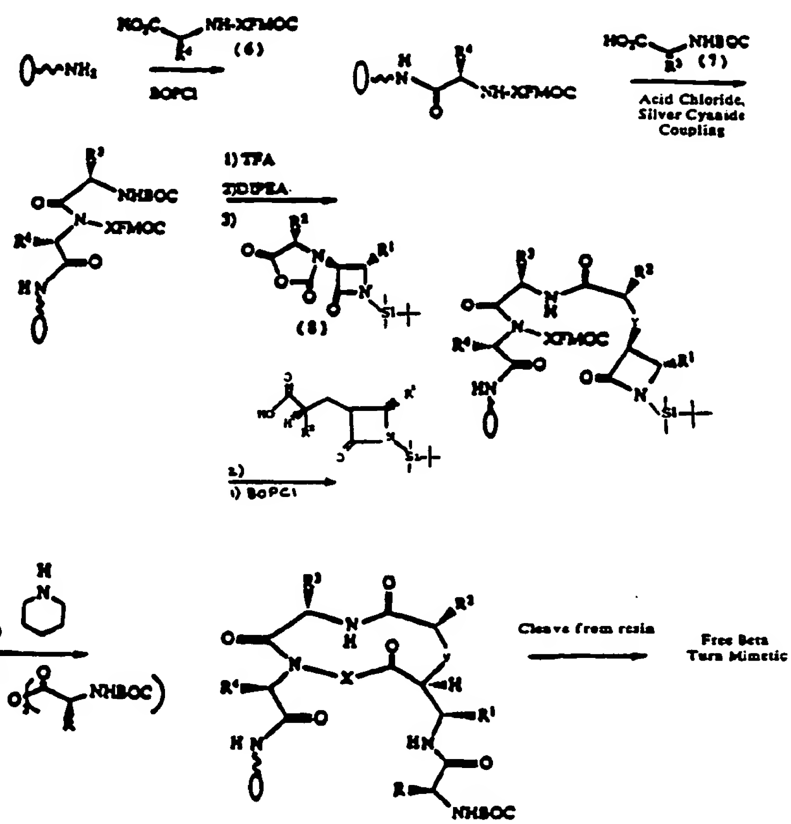
(54) Title: CONFORMATIONALLY RESTRICTED MIMETICS OF BETA TURNS AND BETA BULGES AND PEPTIDES CONTAINING THE SAME

(57) Abstract

The invention provides materials and methods for synthesizing novel beta-turn mimetics, as well as the novel beta-turn mimetics themselves, and peptides containing the same. Also provided are novel synthetic nonpeptide therapeutic molecules designed upon the interactions between beta-turn mimetics or peptides containing the same, and receptors or enzymes.



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CONFORMATIONALLY RESTRICTED MIMETICS OF BETA TURNS AND BETA BULGES AND PEPTIDES CONTAINING THE SAME

BACKGROUND OF THE INVENTION

This invention was supported in part by National Science Foundation Grant CHE-8657046 and National Institute of Health Grant GM38260.

1. Field of the Invention

The invention relates to peptide mimetics, which are chemical structures which serve as appropriate substitutes for peptides or proteins in interactions with receptors, enzymes, and antibodies. The invention also relates to means for analyzing specific interactions between peptides, enzymes, antibodies or receptors, as well as for developing new diagnostic, prophylactic, and therapeutic agents, through the use of peptide mimetics.

2. Summary of the Related Art

Peptides and proteins play critical roles in the regulation of all biological processes. Peptides, for example, play a regulatory role as hormones and inhibitors, and are also involved in immunological recognition. The significant biological role of peptides makes important the understanding of the interactions between peptides and their receptors or enzymes to which they bind.

The determination of the receptor-bound conformation of a peptide is invaluable for the rational design of peptide analogues. However, Marshall et al., Ann. Rep. Med. Chem. 13: 227-238 (1978), discloses that peptides are characteristically highly flexible molecules, the structures of which are strongly influenced by the environment in which they reside. Thus peptides are not generally useful for determining their receptor-bound conformation.

As no approach is available to predict a priori which new ligand-receptor interactions will lead to antagonists and which will lead to agonist of greater or less potency, it is necessary to perform classical structure-function studies in a systematic way to provide information about the specific amino acid residues and functional groups in a peptide that are important to biological activity. Studies of this nature can utilize conformationally constrained peptide mimetics. For example, Hruby, Trends Pharmacol. Sci. 8: 336-339 (1987), suggests that conformational constraints can provide information about the different requirements that a receptor has for a ligand to be an agonist or

antagonist.

Generally, peptide mimetics can be defined as structures which serve as appropriate substitutes for peptides in interactions with receptors and enzymes. The development of rational approaches for discovering peptide mimetics is a major goal of medicinal chemistry. Such development has been attempted both by empirical screening approaches and by specific synthetic design.

Screening of pure chemical entities has been of quite limited utility for discovering peptide mimetics. However, Chipkin et al., Ann. Rep. Med. Chem. 23: 11 (1988), discloses discovery of ligands for the mu-opioid receptor by this approach; as does Romar et al., Nature 298: 760 (1982), for the kappa-opioid receptor.

Screening of complex mixtures of natural products has generally been more successful, especially for the discovery of peptidase inhibitors. For example, Ferreira et al., Biochemistry 9: 2583 (1970), discloses the discovery of the ACE inhibitor, teprotide, by screening the venom of Bothrops jaraca. This approach may also be applied to the discovery of receptor ligands. Chang et al., Science 230: 177 (1985), discloses the discovery of the CCK antagonist asperlicin, using this approach.

Specific design of peptide mimetics has utilized both peptide backbone modifications and chemical mimics of peptide secondary structure. Spatola, Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. VII (Weinstein, Ed.) Marcel Dekker, New York (1983), p. 267, exhaustively reviews isosteric amide bond mimics which have been introduced into biologically active peptides. The beta-turn has been implicated as an important site for molecular recognition in many biologically active peptides. Consequently, peptides containing conformationally constrained mimetics of beta-turns are particularly desirable. Such peptides have been produced using either external or internal beta-turn mimetics.

External beta-turn mimetics were the first to be produced. Friedinger et al., Science 210: 656-658 (1980), discloses a conformationally constrained nonpeptide beta-turn mimetic monocyclic lactam that can readily be substituted into peptide sequences via its amino and carboxy termini, and that when substituted for Gly^6-Leu^7 in luteinizing hormone releasing hormone (LHRH), produces a potent agonist of LHRH activity.

Monocyclic lactams have generally been useful as external beta-turn mimetics for studying receptor-peptide interactions. However, the mimetic

skeleton in these molecules is external to the beta-turn, which gives rise to numerous limitations. Chief among these is bulkiness, which requires the use of dipeptide mimetics, rather than mimetics of all four residues in an actual beta-turn. Substantial flexibility retained in these beta-turn mimetics makes it unsafe to assume that expected conformations are present, absent considerable conformational analysis. For example, Vallee et al., Int. J. Pept. Prot. Res. 33: 181-190 (1989), discloses that a monocyclic lactam beta-turn mimetic did not contain an expected type II' beta-turn in its crystal structure. Another limitation of the monocyclic lactam beta-turn mimetics arises from the difficulty of producing molecules that effectively mimic the side chains of the natural peptide. These difficulties arise from steric hindrance by the mimetic skeleton, which results in a more effective mimic of the peptide backbone than of the side chains. Considering the great importance of side chains in receptor binding, these difficulties strongly limit the versatility of monocyclic lactams.

Although the use of bicyclic lactams reduces problems of flexibility somewhat, conformational analysis of peptides containing these mimetics may still be desirable. Moreover, the side chain hindrance in these molecules may be even worse than that in the monocyclic lactams. Finally, both monocyclic and bicyclic lactams mimic only type II and type II' beta-turns, whereas type I and type III beta-turns are more prevalent in proteins and presumably in peptides.

The limitations presented by external beta-turn mimetics may be minimized by using mimetics in which the mimetic skeleton approximately replaces the space that was occupied by the peptide backbone in the natural beta-turn. Such molecules are known as internal beta-turn mimetics. Internal beta-turn mimetics may not generally reproduce the geometry of the peptide backbone of the particular beta-turn as accurately as external beta-turn mimetics. However, the internal position of the constraint allows replacement of larger sections of peptide, thus making tetrapeptide mimetics possible. The lack of bulk also diminishes the likelihood of steric hindrance of the side chains by the mimetic skeleton.

Internal beta-turn mimetics having biological activity are known in the art. For example, Krstenansky et al., Biochem. Biophys. Commun. 109: 1368-1374 (1982), discloses a leucine enkephalin analog in which an internal beta-turn mimetic replaced the residues Gly^2-Gly^3-Phe^4-Leu^5, and which acted as an analgesic with one-third the potency of morphine. Other internal beta-turn mimetics have been described.

Kahn et al., Tetrahedron Lett. 27: 4841-4844 (1986), discloses an internal beta-turn mimetic, based upon an indolizidinone skeleton, and designed to mimic the lysine and arginine side-chain disposition of the immunosuppressing tripeptide Lys-Pro-Arg.

Kahn et al., Heterocycles 25: 29-31 (1987), discloses an internal beta-turn mimetic, based upon an indolizidinone skeleton, and designed to correctly position the aspartyl and arginyl side chains of a beta-turn in the proposed bioactive region of erabutoxin.

Kahn et al., Tetrahedron Lett. 28: 1623-1626 (1987), discloses a type I beta-turn mimetic which can be incorporated into a peptide via its amino and carboxy termini, and which is designed to mimic an idealized type I beta-turn. See also Kahn et al., J. Am. Chem. Soc. 110: 1638-1639 (1988); Kahn et al., J. Mol. Recogn. 1: 75-79 (1988).

Similarly, Kemp et al., Tetrahedron Lett. 29: 5057-5060 (1988), discloses a type II beta-turn mimetic which can be incorporated into a peptide via its amino and carboxy termini.

Arrhenius et al., Proc. Am. Peptide Symp., Rivier and Marshall, Eds., Escom, Leiden (1990), discloses substitution of an amide-amide backbone hydrogen bond with a covalent hydrogen bond mimic to produce an alpha-helix mimetic.

Diaz et al., Tetrahedron Lett. 32: 5725-28 (1991) discloses a method used to prepare conformationally restricted amino acids which are potential nucleators for the formation of antiparallel and parallel beta-sheet structures.

Thus, there have been numerous successes in obtaining mimetics which can force or stabilize peptide secondary structure. However, little success has been reported in incorporating mimetics at the active site of a peptide hormone or neurotransmitter, probably because of the difficulty of producing mimetics that possess appropriately positioned side chain groups. There is therefore, a need for improved mimetics having greater substituent flexibility to allow for easy synthesis of mimetics having appropriately positioned side chain groups. Moreover, there is a need for improved mimetics having more readily controllable skeletal sizes and angles, so that different types of beta-turn structures can be easily imitated. An ideal mimetic would provide ready control and variation of both side chain positioning and mimetic skeleton size and angles through a modular construction system that allows easy synthesis of a wide variety of mimetics.

For recent reviews of the related art, see Hruby et al., Biochem. J. 268: 249-262 (1990); Ball et al., J. Mol. Recogn. 3: 55-64 (1990); Morgan et al., Ann. Rep. Med. Chem. 24: 243-252 (1989); and Fauchere, Adv. Drug Res. 15: 29-69 (1986).

BRIEF SUMMARY OF THE INVENTION

The invention provides materials and methods for the synthesis of beta-turn mimetics. More particularly, the invention provides a modular system for synthesizing beta-turn mimetics having nearly infinite variability in degree of conformational constraint, flexibility, side chain constituents, and in the size and bond angles of the mimetic skeleton. The materials and methods of the invention are readily amenable to incorporation in conventional and multiple peptide synthesis procedures.

In a first aspect, the invention provides modular component pieces for the assembly of beta-turn mimetics. In a second aspect, the invention provides methods for making the beta-turn mimetics and for making peptides containing the same. In a third aspect, the invention provides novel beta-turn mimetics and novel peptide structures containing such beta-turn mimetics. In a fourth aspect the invention provides novel synthetic nonpeptide diagnostic, prophylactic, and therapeutic molecules.

The materials and methods of the invention are useful for probing the molecular interactions between ligands and receptors, and thus for providing therapeutic agonists and antagonists capable of interacting with receptors, antibodies, or enzymes.

Additional preferred embodiments of the invention will be made apparent by the following detailed description, examples, and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

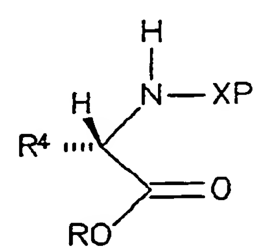
Figures 1 and 2 show routes for synthesizing either a beta-turn mimetic according to the invention, or a novel peptide containing the same. The synthesis route shown in Figures 1 and 2 utilizes the modular component pieces of the invention in a standard Merrifield synthesis scheme to produce a beta-turn mimetic.

Figure 3 shows preferred embodiments of the linker moiety, X, of the first modular component piece. For each linker shown, n = 0-4 and R = H or CH_3. Aromatic linkers are shown in para configuration, but may alternatively be in ortho or meta configuration.

DETAILED DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The invention provides a modular system for producing beta-turn mimetics having a virtually limitless range of skeletal sizes and bond angles, and side chain substituents. Beta-turn mimetics according to the invention can thus have changed side chain substituents without having any changes in the backbone conformation. Moreover, beta-turn mimetics according to the invention possess appropriate termini for incorporation into peptides by standard peptide synthesis procedures. Thus the invention provides a system for producing a virtually unlimited array of peptides having beta-turn mimetics according to the invention incorporated therein. For purposes of the invention the term "beta turn mimetics" is sometimes used in a general sense, and is intended to encompass mimetics of beta turns, gamma turns, beta hairpins, and beta bulges, all of which are provided by the invention by varying the modular component pieces used.

In a first aspect, the invention provides modular component pieces for the construction of beta-turn mimetics. Modular component pieces according to the invention include both L- and D-enantiomeric forms. A first modular component piece according to the invention is characterized by the structural formula



wherein R^4 may be any naturally-occurring amino acid side chain substituent, or analog thereof, wherein P is a protective group suitable for use in peptide synthesis, and wherein the linker moiety, X comprises a linker terminating in an amino or hydrazino group, and wherein the termini of the linker are separated by zero to ten carbon atoms, and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic. Specific preferred examples of such linkers are shown in Figure 2.

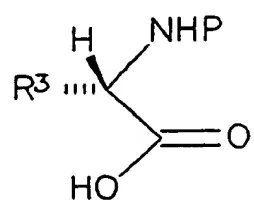
The linker group X may be varied in size and or flexibility to control the conformation of the ring in the final mimetic. This allows the construction in a predictable fashion of a nearly infinite variety of conformationally restricted

ligands. Ligands having maximum biological activity can then be subjected to spectroscopic and computer-assisted molecular modeling to infer the bound conformation from the determined solution structure.

Such first component piece may be synthesized according to alternative routes, depending on the nature of the X groups. According to a first route, as shown in Figure 1, the component is synthesized by the SN2 displacement of an alpha-triflyloxy ester which is readily produced from the corresponding amino acid according to the procedure described by Hoffman and Kim, Tetrahedron Lett. 31: 2953 (1990) or by the direct amination procedure of Vidal, JCS Chem. Comm. 435 (1991).

An alternative route for synthesis of the first component piece is shown in Figure 2 and utilizes a quite facile reductive amination reaction, as described by Gribble and Nutaitis, Org. Prep. Proced. Int. 17: 317, 1985 and Sasaki and Coy, Peptides 8: 119 (1987). This method has the advantage of being readily amenable to a large variety of aldehyde components, thus providing a large array of X linker moieties.

A second modular component piece according to the invention comprises an N-protected naturally occurring amino acid or analog thereof, and may be represented by the structural formula

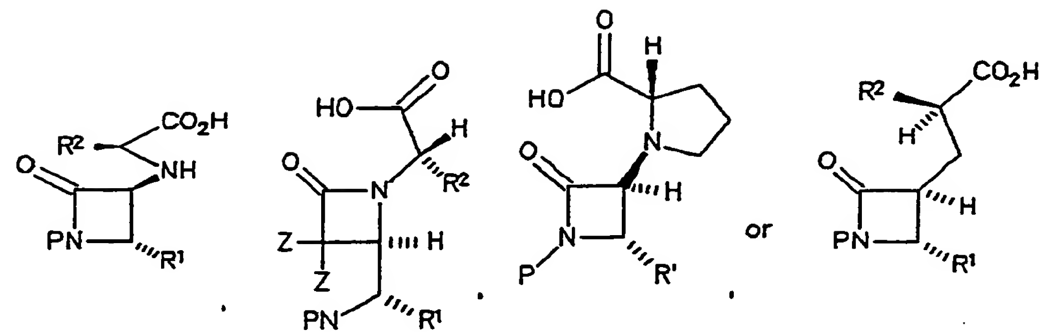


wherein P is a protective group suitable for use in peptide synthesis, and wherein R^3 = a naturally-occurring amino acid side chain or analog thereof. A completed mimetic may contain none, one, or more second modular component pieces. When more than one second modular component piece is present in a mimetic, additional R groups will be represented in structural formulae as $R^{3'}$.

A third modular component piece according to the invention is characterized by the structural formula

wherein P is a protective group suitable for use in peptide synthesis, wherein Z = H or CH_3, and wherein R^1 and R^2 = naturally-occurring amino acid side chains or analogs thereof. Preferred protective groups include a tert-butyl dimethylsilyl group, or butoxycarbonyl (BOC).

Such a third modular component piece according to the invention may



be synthesized by the routes shown in Examples 6-8, which entails selective generation of the exocyclic enolate and condensation with an appropriate N-silylimine, followed by mild hydrolysis. See Hart and Hu, Chem. Rev. 89: 1447 or Salzman et al., J. Am. Chem. Soc. 102: 6161 (1980); Miller et al., J. Am. Chem. Soc. 102: 7026 (1980); Williams et al., J. Amer. Chem. Soc. 111: 1073 (1989).

In another aspect, the invention provides a method for producing beta-turn mimetics, comprising generally the steps shown in Figure 1. Typically, a free amino group coupled to a solid support will be the starting point of the synthesis. The amino group may be coupled to the solid support via a nonpeptide chemical constituent, or it may be the free amino terminus of a nascent peptide being synthesized from the solid support. A first modular component piece according to the invention is coupled via an amide linkage to the free amino group bound to the solid support, to yield a support-bound first modular component piece. A second modular component piece according to the invention is then coupled to the support-bound first modular component piece, using an acid chloride, silver cyanide coupling reaction as shown in Tung, et al., J. Org. Chem., 51: 3350, (1986), to yield a support-bound nascent beta-turn mimetic. A mixed anhydride coupling is then carried out between a third modular component piece and the support-bound nascent beta-turn mimetic to yield a support-bound pre-cyclization beta-turn mimetic which is then cyclized to form a support-bound beta-turn mimetic. At this point peptide synthesis may be continued, or the support-bound structure may be cleaved from the support.

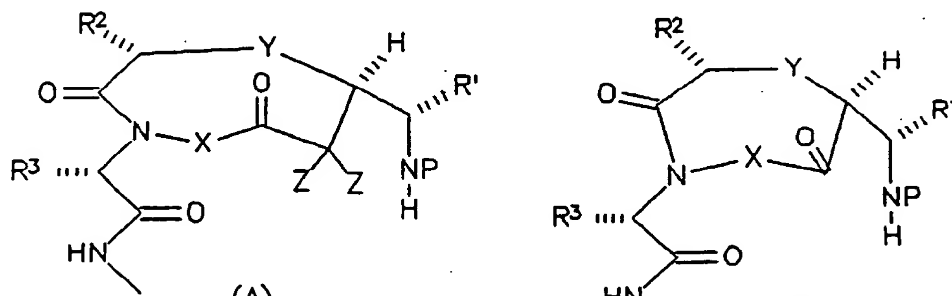
Yet another route to producing a beta turn mimetic is shown in Figure 2. Again a free amino group coupled to a solid support is the starting point for the synthesis. A first modular component piece, including linker X, bound to a protective group according to the invention, is coupled via amide linkage to the free amino group bound to the solid support to yield a support-bound first modular component piece. The linker X of the support-bound first modular

component piece is deprotected. Then the four member ring of a third modular component piece is opened nucleophilically and coupled to the deprotected support-bound first modular component to yield a support bound nascent beta-turn mimetic. The support-bound nascent beta-turn mimetic is then coupled with a doubly protected naturally occuring amino acid or analog thereof, as shown in Bambino et al., Tetrahedron Letters, 32; 3407-8, 1991, to yield a support-bound pre-cyclization beta-turn mimetic. The final reaction steps are essentially equivalent to those discussed in conjunction with Figure 1.

Synthesis of beta-turn mimetics may be carried out in solution. Synthesis in solution requires essentially the same steps as solid-phase synthesis except that the first modular component piece is not attached to a solid support.

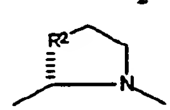
Those skilled in the art will recognize the methods of this invention may be used to synthesize an isolated beta-turn mimetic having variable side chain constituents and backbone size and bond angles, or that it may be readily incorporated into standard Merrifield solid phase peptide synthesis to produce a peptide having such a beta-turn mimetic within it or at either end.

"Beta-turn mimetics" according to the invention actually encompass mimetics of related structures, including gamma turns, beta turns, and beta bulges. Examples of mimetic gamma turns according to the invention include those represented by the structural formulae



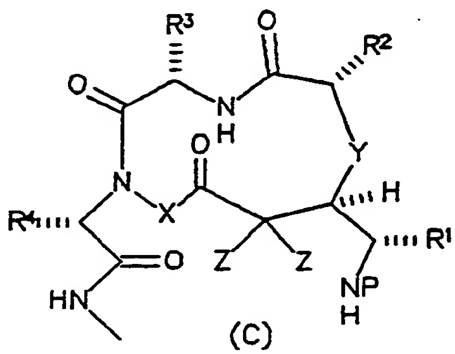
(A) (B)

wherein Z = H or CH_3, and Y = CH_2, NH, NCH_3 or

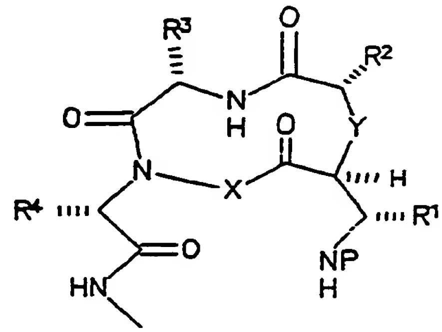


Gamma turn mimetics according to the invention are prepared by directly linking together first and third modular component pieces without the use of a second modular component piece.

Mimetics of actual beta-turns, according to the invention, include those represented by the structural formulae

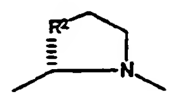


(C)



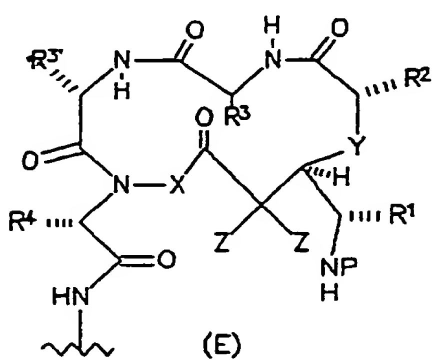
(D)

wherein Y = CH_2, NH, $NHCH_3$ or

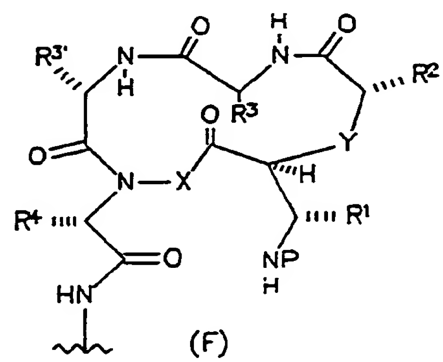


and Z = H or CH_3.

Examples of beta-bulge mimetics according to the invention include the following stuctures

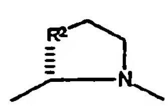


(E)



(F)

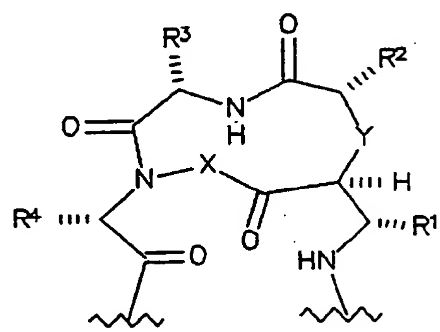
wherein Y = CH_2, NH, $NHCH_3$ or and Z = H, CH_3 or



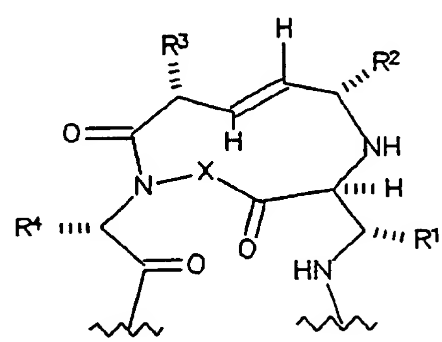
Beta-bulge mimetics according to the invention are prepared by linking two second modular component pieces between the first and third modular component pieces.

In all "beta-turn mimetics", according to the invention, X = a linker group selected from the group described previously.

Thus, in a third aspect, the invention provides both beta-turn mimetics having variable sizes and bond angles and variable side chain constituents, and peptides containing such beta-turn mimetics internally or at either end. Such beta-turn mimetics, or peptides containing the same, are conformationally restricted, and as such are useful for the design and synthesis of conformationally restricted antigens for making synthetic vaccines or for making antibodies for diagnostic purposes. Additionally, they are useful for mapping critical receptor-ligand interactions for purposes of designing nonpeptide therapeutics. They are useful not only for initial mapping, based upon which beta-turn mimetics bind the receptor, but are also useful for subsequent investigation directed toward identification of molecular interactions critical to the binding. For example, if a beta-turn mimetic represented by the structural formula



was found to bind to a receptor of interest, the significance of particular hydrogen bonds in its binding can easily be tested by preparing an analog of the beta-turn mimetic that cannot form these bonds, for example



Synthetic nonpeptide molecules can then be produced based upon information obtained from nuclear magnetic resonance (NMR) to determine binding interactions and bound-state conformations of these structures; and employing molecular modeling to interpret the NMR data and to predict

improved synthetic nonpeptide structures.

NMR conformational analysis for small peptide and peptide analog systems in solution is straightforward and well known in the art. For example, see Bax, Two-Dimensional Nuclear Magnetic Resonance in Liquids, D. Reidel Publishing Co., Boston, 1982; Wuthrich, NMR of Proteins and Nucleic Acids, Wiley-Interscience, New York, 1986; Ernst et al., Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Oxford University Press, New York, 1987.

NMR along with computer-assisted molecular modeling allows the identification of ligand-receptor interactions required for binding. Identifying the interactions required for binding facilitates preparation of synthetic molecules that are capable of similar binding, and therefore of acting as agonists or antagonists. Once a single stable binding conformation is known, the design and preparation of a synthetic therapeutic molecule capable of acting as an agonist or antagonist is thus brought within the ability of one skilled in the art, without requiring undue experimentation.

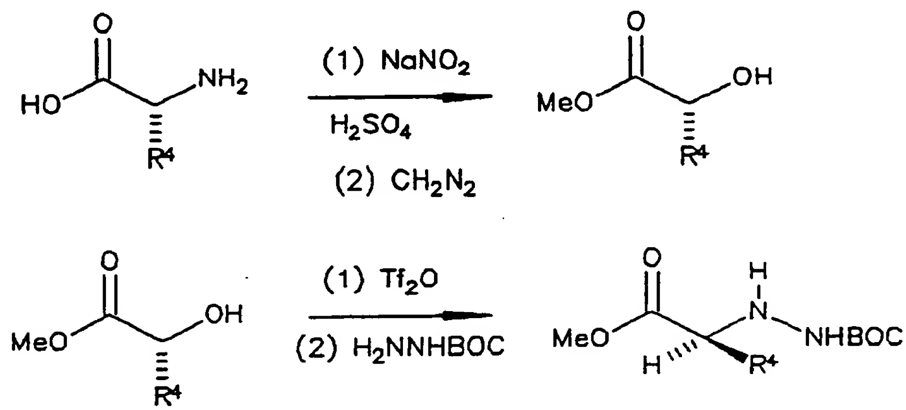
Thus, in a fourth aspect, the invention provides synthetic therapeutic molecules capable of acting as agonists or antagonists, wherein such molecules are based upon structural features of a conformationally restricted beta-turn mimetic that is capable of binding to the receptor. Particularly likely candidates for the development of such therapeutics include synthetic molecules based upon one or more structural features of a binding conformation of a peptide hormone, lymphokine, growth factor, enzyme inhibitor, or viral binding protein.

The following examples are intended to further illustrate the invention, and are not limiting in nature.

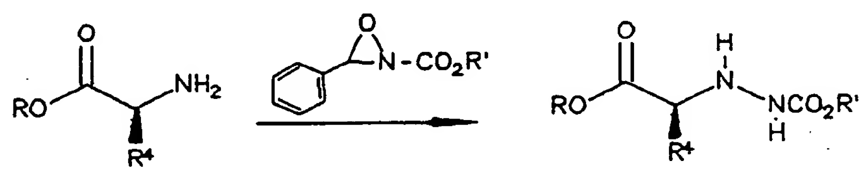
Example 1

Synthesis of a First Modular Component Piece

First modular component pieces were synthesized according to the following schemes.



See Hoffman and Kim, Tetrahedron Lett. 31: 2953 (1990), and;

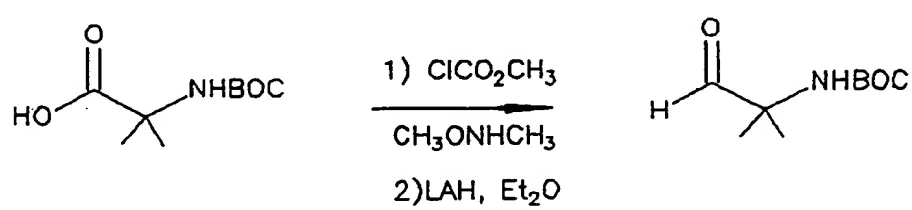


See Vidal, J. Chem. Soc. Chem. Commun. 435 (1991).

Example 2

Aldehyde Synthesis from Corresponding Carboxylic Acid

Aldehydes were synthesized from their corresponding carboxylic acids according to the following scheme.

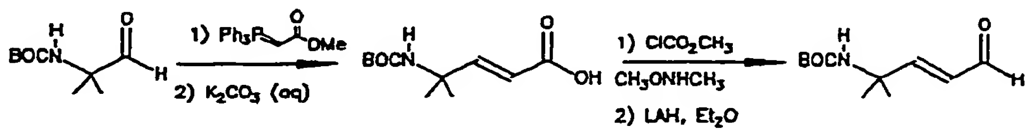


See Goel et al., Org. Syn. 67: 69 (1988).

Example 3

Wittig Reaction Homologation of Aldehydes

Homologation of aldehydes was carried out using the Wittig reaction, according to the following scheme.

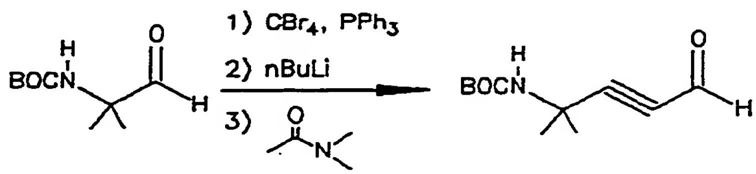


See House and Rasmusson, J. Org. Chem. 26: 4278 (1961).

Example 4

Alternative Homologation of Aldehydes

Homologation of aldehydes was alternatively carried out according to the following scheme.

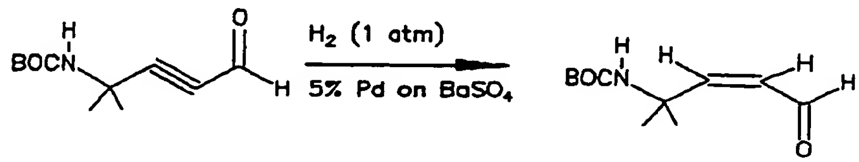


See, Tetrahedron Lett. 13: 3769 (1972).

Example 5

Cis-Olefin Synthesis by Lindlar Reduction of Acetylene

Acetylenes prepared according to Example 4 were used in the Lindlar reduction to prepare cis-isomers.

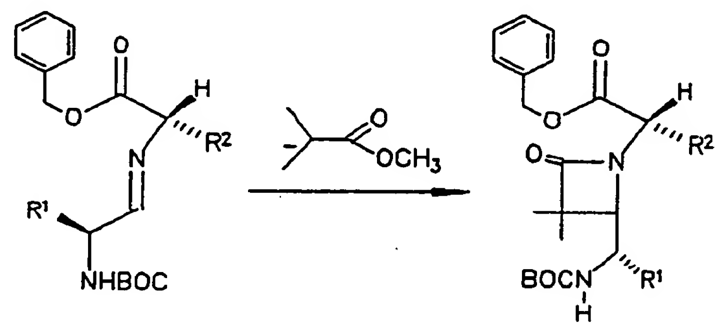


See Lindlar, Helv. Chim. Acta 35: 446 (1952). For reductive animation, see Gribble et al., Organic Prep. Proced. Int. 17: 317 (1985).

Example 6

Synthesis of Third Modular Component Pieces

Third modular component pieces were synthesized according to the following scheme.

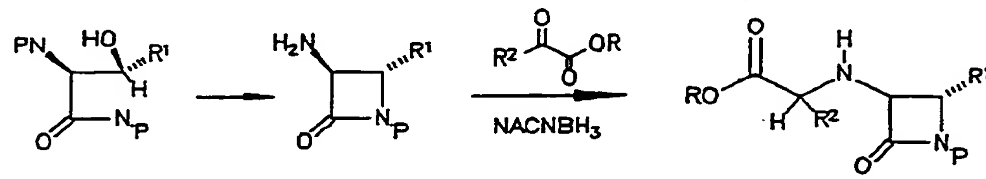


See Hart and Hu, Chem. Rev. 89: 1447 (1990). Third modular component pieces synthesized according to this example are used to create mimetics wherein R^2 is attached to a carbon atom adjacent to a tertiary nitrogen.

Example 7

Alternative Synthesis of Third Modular Component Pieces

Third modular component pieces were alternatively synthesized according to the following scheme.

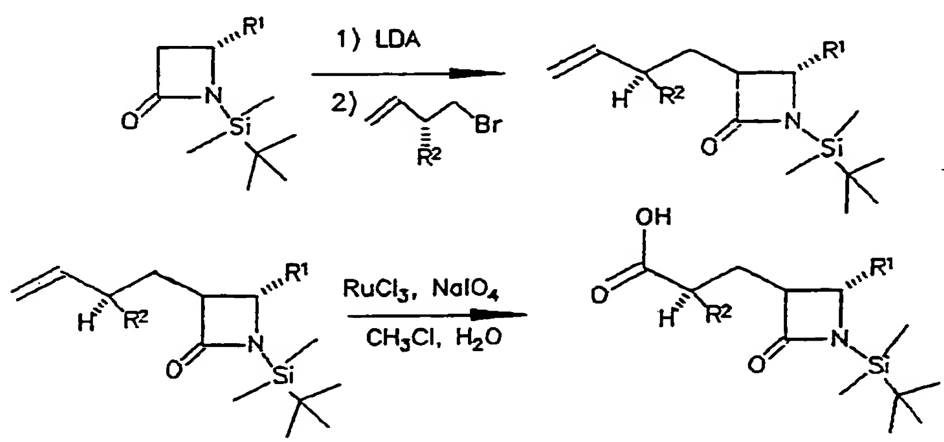


See Miller et al., J. Am. Chem. Soc. 102: 7026 (1980). Third modular component pieces synthesized according to this example are use to create mimetics having R^2 attached to a carbon atom adjacent to a secondary nitrogen atom.

Example 8

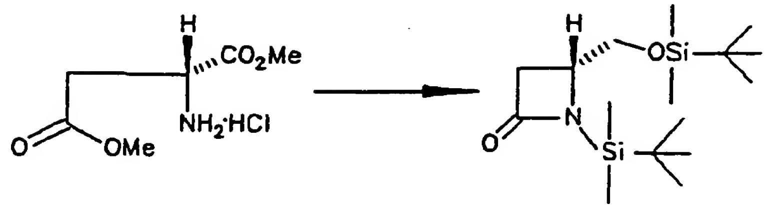
Additional Alternative Synthesis of Third Modular Component Pieces

Third modular component pieces were further synthesized according to the following scheme.



See Williams et al., J. Amer. Chem. Soc. 111: 1073 (1989). Third modular component pieces synthesized according to this example are used to create mimetics having R^1 attached to a carbon atom adjacent to a secondary or tertiary nitrogen atom.

Example 9

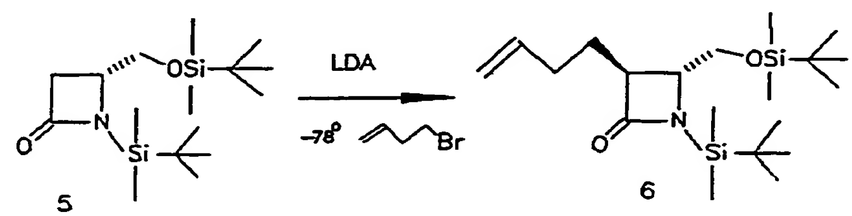


Preparation of N-t-butyldimethylsilyl-4-(R)-t-butyldimethyl-silyloxy-2-azetidinone 1

D-aspartic acid dimethylester hydrochloride (2.00 g, 10.1 mmol), t-butyldimethylsilyl chloride (1.68 g, 11.1 mmol) and 4-dimethylaminopyridine (62 mg. 0.51 mmol) were dissolved in 50 ml of methylene chloride. To this mixture was added triethylamine (3.24 ml, 23.3 mmol) at room temperature slowly and the mixture was allowed to stir overnight at room temperature. The mixture was washed with aqueous ammonium chloride, saturated sodium bicarbonate and brine, dried over sodium sulfate and concentrated in vacuo. The residue was dissolved in 50 ml of ether. The solution was cooled to 0°C and 2.0 M t-butylmagnesium chloride in ether (5.24 ml, 10.5 mmol) was added dropwise. The mixture was allowed to warm to room temperature overnight with stirring and was coooled to 0°C again. Saturated ammonium chloride was added and the mixture was stirred for 30 min. Water was added to the mixture and the organic layer was separated. The aqueous layer was extracted with ether (2x30 ml). The combined organic extracts were washed with brine, dried over magnesium sulfate and concentrated in vacuo. The residue was dissolved in 60 ml of methanol. To this solution at room temperature, sodium borohydride (1.14 g, 30.1 mmol) was added to a flask equipped with a reflux condenser. The mixture began to reflux during the addition and ceased after 20 min. After 45 min. in total, the mixture was cooled to 0°C and aqueous ammonium chloride was added. The mixture was extracted with methylene chloride (3x50 ml). The combined organic extracts were dried over sodium sulfate and the volatiles were removed in vacuo. The residue was dissolved in 30 ml of methylene chloride. To this solution was added t-butyl-dimethylsilyl chloride (1.00 g, 6.63 mmol) and 4-dimethylamino-pyridine (37 mg, 0.30 mmol). Triethylamine (1.10 ml, 7.87 mmol) was added slowly and the mixture was allowed to stir overnight at r.t. The mixture was washed with aq. ammonium chloride and brine, dried over sodium sulfate and concentrated in vacuo. Flash chromatography of the residue on

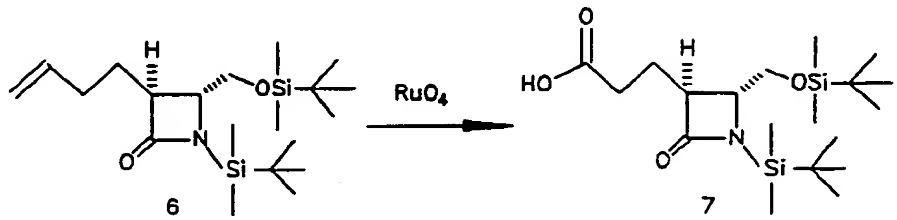
silica-gel with hexane-ethyl acetate (9/1:v/v) afforded 1.01 g (30%) of 1 as a colorless liquid. H NMR (400 MHz, $CDCl_3$ delta 3.74 (dd, J_a=3.96 Hz, J_b=10.30 Hz, 1H), 3.63 (dd, J_a=5.12 Hz, J_b=10.30 Hz, 1H), 3.59 (m, 1H), 3.04 (dd, J_b=5.28 Hz, J_b=15.22 Hz, 1H), 2.76 (dd, J_a=2.49 Hz, J_b=15.22 Hz, 1H), 0.94 (s, 9H), 0.88 (s, 9H), 0.22 (s, 3H), 0.21 (s, 3H), 0.05 (S, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): delta 172.7, 65.3, 50.2, 41.2, 26.2, 25.8, -5.4, -5.5, -5.7.

Example 10



A solution of lithium diisopropyl amide (2.5 mmol in 25 ml of THF) was prepared in the usual manner at 0°. After cooling to -78°C, a solution of azetidinone 5 (323 mg, 1 mmol) in 10 ml of THF was added dropwise and allowed to stir for 30 minutes at -78°C. To this was added 400 ml (4 mmol) of butenyl bromide. Stirring was continued for 18 h. and the reaction allowed to come to room temperature. The reaction mixture was poured into saturated NH_4Cl solution and extracted 3 times with 50 ml portions of ether, dried over Na_2SO_4 and the solvent removed in vacuo. The residue was chromatographed on 15 g of silica gel to provide 294 mg, 78% of azetidinone 6.

Example 11



A flask was charged with a magnetic stirrer, $CCl_4/CH_3CN/H_2O$ (1:1:2, total 4 ml), azetidinone 6 (160 mg, 0.44 mmol) and $NaIO_4$ (469 mg. 2.2 mmol, 5eq). To this biphasic solution, a catalytic amount of $RuCl_3$ $3H_2O$ was added. The mixture was stirred overnight at room temperature and taken up in ethyl acetate (25 ml) and H_2O (10 ml). The organic layer was separated and the aqueous layer was saturated with sodium chloride (solid) and extracted with ethyl acetate (2 x 20 ml). The combined organic extracts were dried over Na_2SO_4 and concentrated to provide 7 as an oil in 55-65% yield.

I CLAIM:

1. A method of producing beta-turn mimetics, or peptides containing beta-turn mimetics, comprising the steps of:

(a) binding a first modular component piece to a solid support, to yield a support-bound first modular component piece;

(b) coupling a second modular component piece to the support-bound first modular component piece by an acid chloride, silver cyanide coupling, to yield a support-bound nascent beta-turn mimetic;

(c) coupling a third modular component piece to the support-bound nascent beta-turn mimetic by a mixed anhydride reaction, to yield a support-bound pre-cyclization beta-turn mimetic;

(d) cyclizing the support-bound pre-cyclization beta-turn mimetic to yield a support-bound beta-turn mimetic; and

(e) cleaving the support-bound beta-turn mimetic from the solid support to yield a beta-turn mimetic.

2. A method of producing beta-turn mimetics, or peptides containing beta-turn mimetics, comprising the steps of:

(a) binding a first modular component piece including a linker to a solid support, to yield a support-bound first modular component piece;

(b) deprotecting the linker;

(c) opening the four-member component ring in a third modular component piece to yield an opened third modular component piece;

(d) coupling the opened third modular component piece to the support-bound first modular component piece by a mixed anhydride reaction to yield a support-bound pre-cyclization beta-turn mimetic;

(e) cyclizing the support-bound pre-cyclization beta-turn mimetic by mixed anhydride coupling to yield a support-bound beta-turn mimetic; and

(f) cleaving the support-bound beta-turn mimetic from the solid support to yield a beta-turn mimetic.

3. A method of producing beta-turn mimetics, or peptides containing beta-turn mimetics, comprising the steps of:

(a) coupling a first modular component piece to a second modular component piece by an acid chloride, silver cyanide coupling to yield a

nascent beta-turn mimetic;

(b) coupling a third modular component piece to the nascent beta-turn mimetic by a mixed anhydride reaction, to yield a pre-cyclization beta-turn mimetic; and

(c) cyclizing the pre-cyclization beta-turn mimetic to yield a beta-turn mimetic.

4. A method of producing gamma-turn mimetics, or peptides containing beta-turn mimetics, comprising the steps of:

(a) binding a first modular component piece to a solid support, to yield a support-bound first modular component piece;

(b) coupling a third modular component piece to the support-bound first modular component piece by an acid chloride, sliver cyanide coupling reaction, to yield a support-bound pre-cyclization gamma-turn mimetic;

(c) cyclizing the support-bound pre-cyclization gamma-turn mimetic to yield a support-bound gamma-turn mimetic; and

(d) cleaving the support-bound gamma-turn mimetic from the solid support.

5. A method of producing gamma-turn mimetics, or peptides containing gamma-turn mimetics, comprising the steps of:

(a) coupling a first modular component piece to a third modular component piece by an acid chloride, silver cyanide coupling reaction, to yield a pre-cyclization gamma-turn mimetic; and

(b) cyclizing the pre-cyclization gamma-turn mimetic to yield a gamma-turn mimetic.

6. A method of producing beta-bulge mimetics, or peptides containing beta-bulge mimetics, comprising the steps of:

(a) binding a first modular component piece to a solid support,.to yield a support-bound first modular component piece;

(b) coupling a second modular component piece to the support-bound first modular component piece by an acid chloride silver cyanide coupling reaction, to yield a support-bound nascent beta-bulge mimetic;

(c) coupling another second modular component piece to the support-

bound nascent beta-bulge mimetic by an anhydride coupling reaction, to yield an extended support-bound nascent beta-bulge mimetic;

(d) coupling a third modular component piece to the extended support-bound nascent beta-bulge mimetic by a mixed anhydride reaction, to yield a support-bound pre-cyclization beta-bulge mimetic;

(e) cyclizing the support-bound pre-cyclization beta-bulge mimetic to yield a support-bound beta-bulge mimetic; and

(f) cleaving the support-bound beta-bulge mimetic from the solid support.

7. A method of producing beta-bulge mimetics, or peptides containing beta-bulge mimetics, comprising the steps of:

(a) coupling a first modular component piece to a second modular component piece by an anhydride coupling reaction, to yield a nascent beta-bulge mimetic;

(b) coupling another second modular component piece to the nascent beta-bulge mimetic by an anhydride coupling reaction, to yield an extended nascent beta-bulge mimetic;

(c) coupling a third modular component piece to the extended nascent beta-bulge mimetic by a mixed anhydride reaction, to yield a pre-cyclization beta-bulge mimetic; and

(d) cyclizing the pre-cyclization beta-bulge mimetic to yield a beta-bulge mimetic.

8. A method of producing beta-turn mimetics or peptides containing beta-turn mimetics, comprising the step of carrying out the chemical reaction scheme shown in Figure 1.

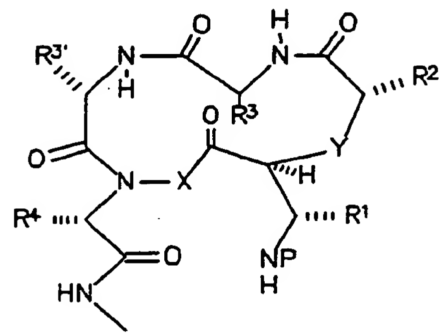
9. A method of producing beta-turn mimetics or peptides containing beta-turn mimetics, comprising the step of carrying out the chemical reaction scheme shown in Figure 2.

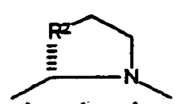
10. A beta-turn mimetic or peptide containing a beta-turn mimetic, produced according to the method of claim 1 or 2.

11. A gamma-turn mimetic or peptide containing a gamma-turn mimetic, produced according to the method of claim 3 or 4.

12. A beta-bulge mimetic or peptide containing a beta-bulge mimetic, produced according to the method of claim 5 or 6.

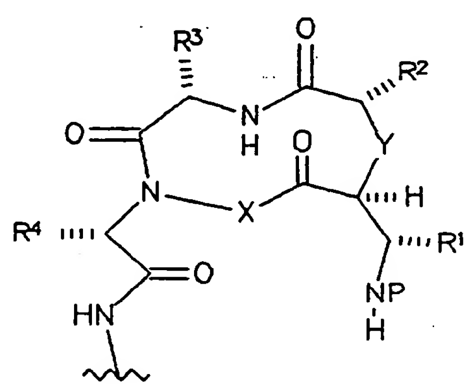
13. A beta-bulge mimetic comprising the structural formula



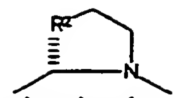
wherein P is a protective group suitable for use in peptide synthesis, wherein R_1-R_4 = naturally-occurring amino acid side chains or analogs thereof, wherein Y = CH_2, NH, NCH_3 or 

wherein X comprises a linker terminating in an amino or hydrazino group, and wherein the termini of the linker are separated by zero to ten carbon atoms and and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic.

14. A beta-turn mimetic comprising the structural formula

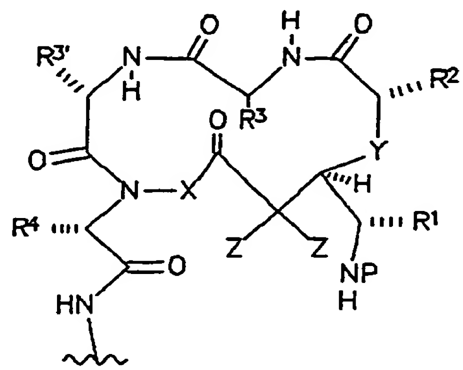


wherein P is a protective group suitable for use in peptide synthesis, wherein R_1-R_4 = naturally-occurring amino acid side chains or analogs thereof,

wherein Y = CH_2, NH, NCH_3 or 

wherein X comprises a linker terminating in an amino or hydrazino group, and and wherein the termini of the linker are separated by zero to ten carbon atoms, and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic.

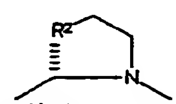
15. A beta-hairpin mimetic comprising the structural formula



wherein P is a protective group suitable for use in peptide synthesis, wherein R_1-R_4 = naturally-occurring amino acid side chains or analogs thereof,

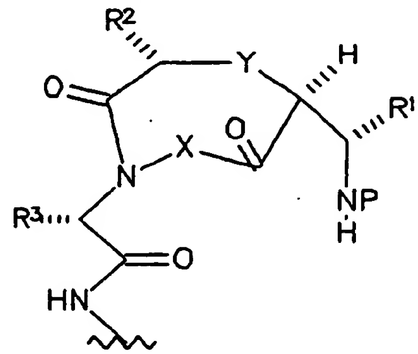
wherein Z = H or CH_3,

wherein Y = CH_2, NH, NCH_3 or

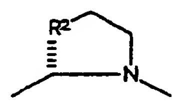


and wherein X comprises a linker terminating in an amino or hydrazino group and wherein the termini of the linker are separated by zero to ten carbon atoms, and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic.

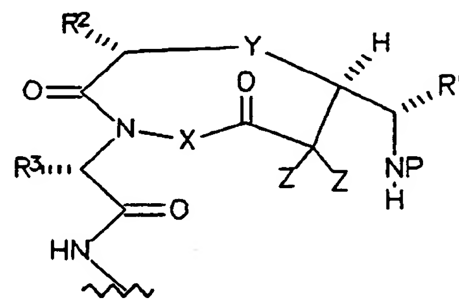
16. A gamma-turn mimetic comprising the structural formula



wherein P is a protective group suitable for use in peptide synthesis, wherein R_1-R_4 = naturally-occurring amino acid side chains or analogs thereof, wherein X comprises a linker terminating in an amino or hydrazino group, and wherein the termini of the linker are separated by zero to ten carbon atoms, and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic and wherein Y = CH_2, NH, NCH_3 or

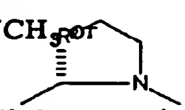


17. A gamma-turn mimetic comprising the structural formula



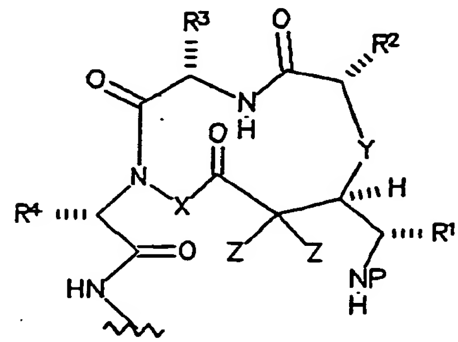
wherein P is a protective group suitable for use in peptide synthesis, wherein R_1-R_4 = naturally-occurring amino acid side chains or analogs thereof,

wherein Z = H or CH_3, and

wherein Y = CH_2, NH, NCH_3, or 

wherein X comprises a linker terminating in an amino or hydrazino group, and wherein the termini of the linker are separated by zero to ten carbon atoms, and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic.

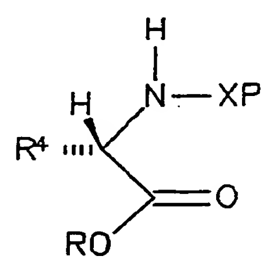
18. A beta-turn mimetic comprising the structural formula



wherein P is a protective group suitable for use in peptide synthesis, wherein R_1-R_4 = naturally-occurring amino acid side chains or analogs thereof, wherein Z = H or CH_3,

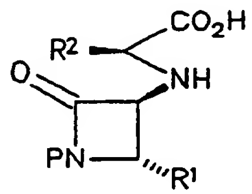
wherein X comprises a linker terminating in an amino or hydrazino group, and wherein the termini of the linker are separated by zero to ten carbon atoms, and wherein the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic.

19. A first modular component piece characterized by the structural formula

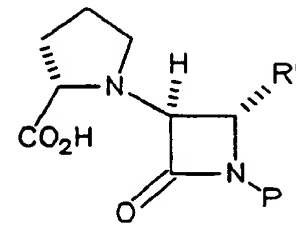


wherein R^4 = a naturally-occurring amino acid side chain or analogs thereof, wherein P is a protective group suitable for use in peptide synthesis, and wherein X comprises a linker terminating in an amino or hydrazino group, and wherein the termini of the linker are separated by zero to ten carbon atoms, and where the carbon atoms involved in carbon-carbon or carbon-nitrogen bonds may be saturated, unsaturated, or aromatic.

20. A third modular component piece characterized by the strucutural formula

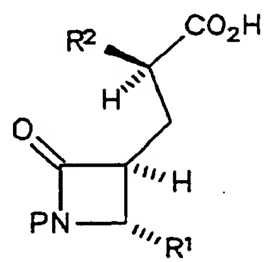


or



wherein R^1 and R^2 represent naturally-occurring amino acid side chains or analogs thereof, and wherein P is a protective group suitable for use in peptide synthesis.

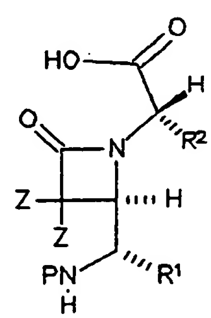
21. A third modular component piece comprising the strucutural formula



wherein R^1 and R^2 represent naturally-occurring amino acid side chains or analogs thereof, and wherein P is a protective group suitable for use in peptide synthesis. (preferably tbutlydimethylsilyl)

22. The third modular component piece of claim 21 wherein P is tert-butyl dimethylsilyl

23. A third modular component piece comprising the strucutural formula



wherein Z represents H or CH_3, wherein R^1 and R^2 represent naturally-occurring amino acid side chains or analogs thereof, and wherein P is a protective group suitable for use in peptide synthesis. (preferably BOC)

24. The third modular component piece of claim 23 wherein P is butoxycarbonyl (BOC).

25. A synthetic nonpeptide therapeutic agent having the ability to bind to a receptor or enzyme, wherein the ability to bind to a receptor or enzyme is by means of structural features deduced from the molecular interactions between the receptor or enzyme and the beta-turn mimetic or peptide containing a beta-turn mimetic of claim 3, 4, or 5, or analogs thereof.

FIGURE 1

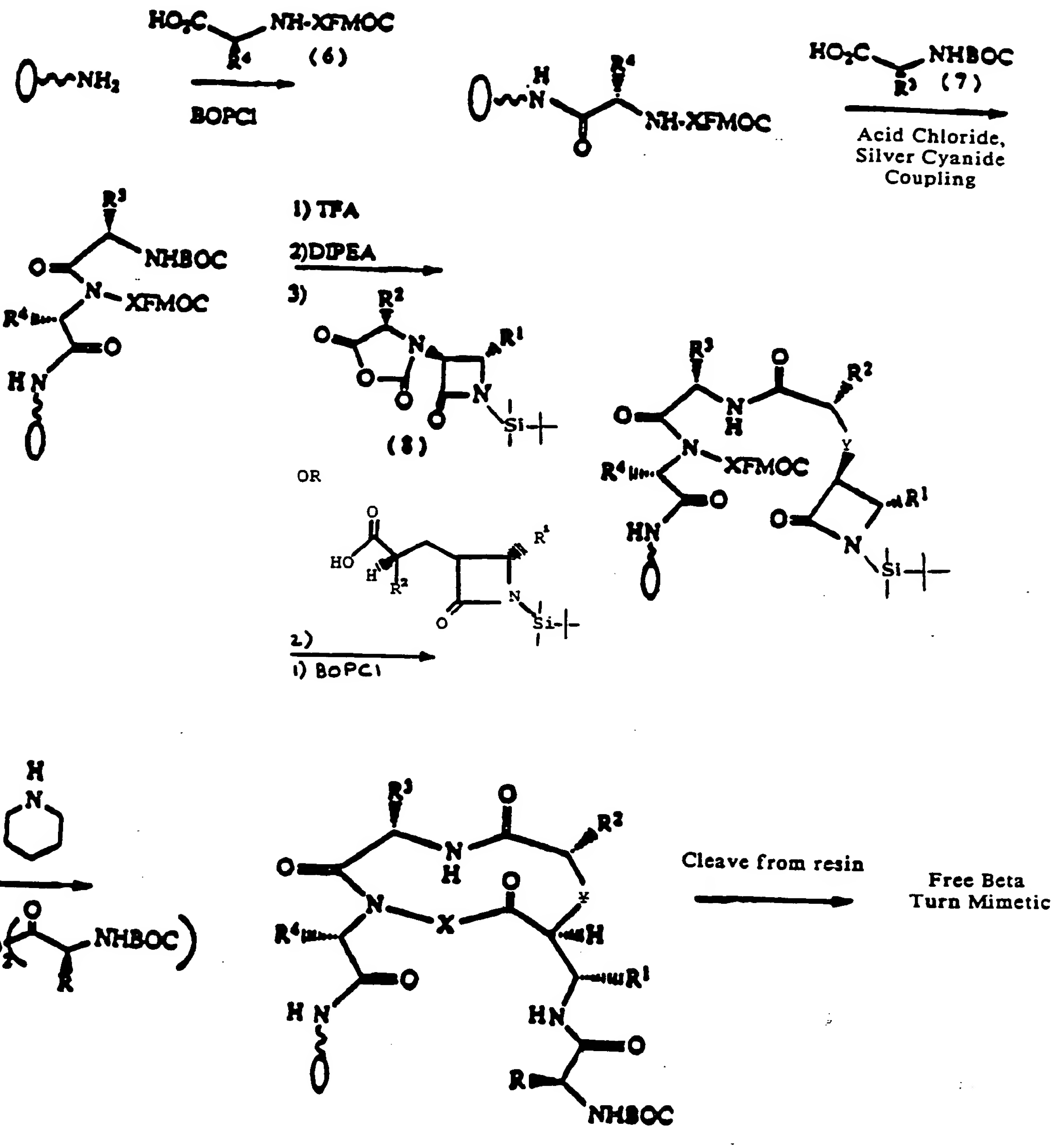

NH₂
HO₂C
NH-XFMOC
R⁴
(6)
BOPCl
H
N
R⁴
O
NH-XFMOC
HO₂C
NHBOC
R³
(7)
Acid Chloride,
Silver Cyanide
Coupling
R³
NHBOC
XFMOC
R⁴
HN
1) TFA
2)DIPEA
3)
R²
R¹
Si
(8)
OR
HO
R²
R¹
Si
2)
1) BoPCl
R³
R²
N
H
XFMOC
R⁴
HN
R¹
Si
1)
H
N
2)
NHBOC
R
R³
R²
N
H
R⁴
X
H
R¹
HN
HN
R
NHBOC
Cleave from resin
Free Beta
Turn Mimetic

FIGURE 2

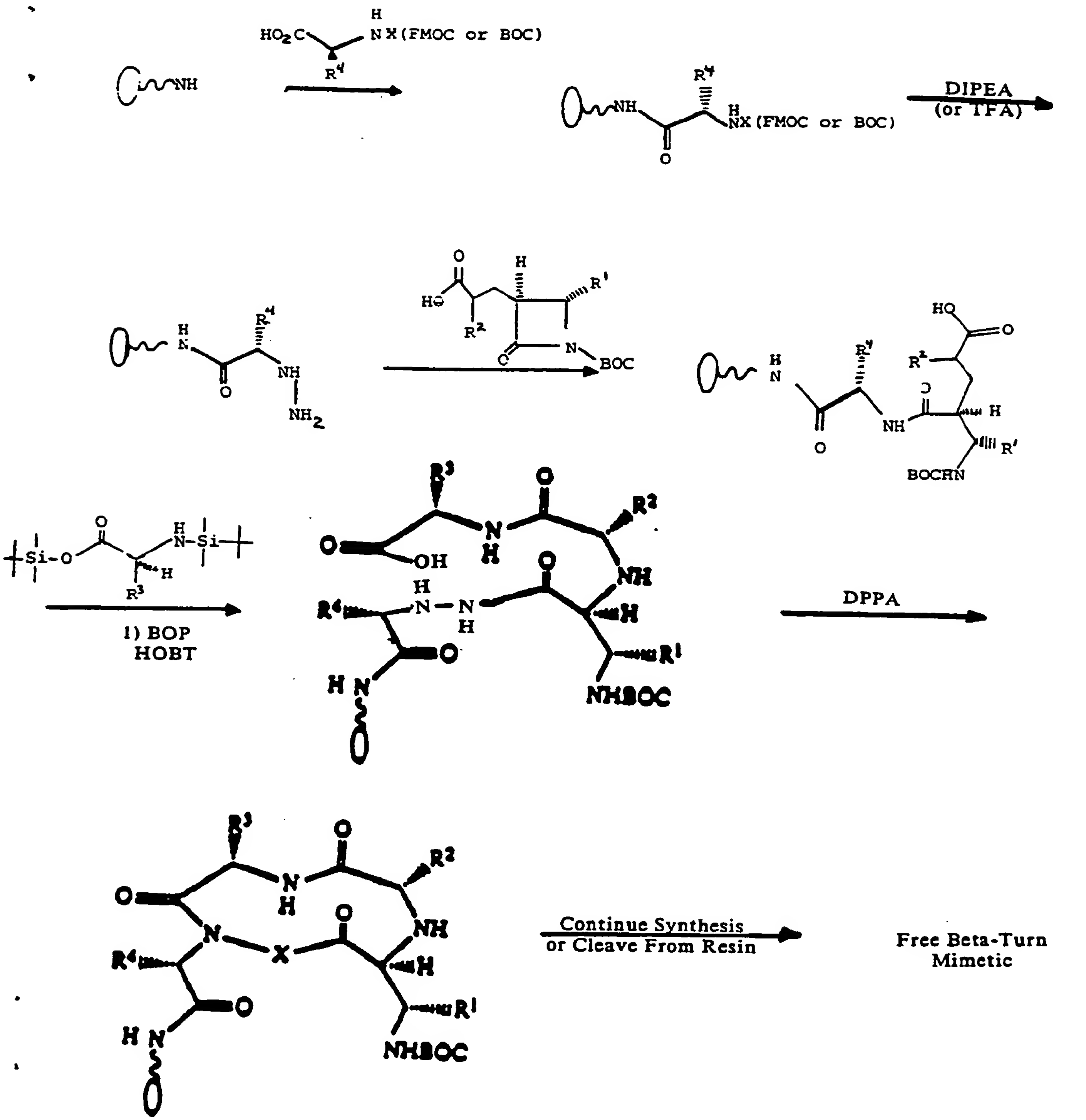

HO2C
NX (FMOC or BOC)
R4
NH
DIPEA
(or TFA)
R2
R1
BOC
HO
BOCHN
1) BOP
HOBT
R3
OH
NHBOC
DPPA
X
Continue Synthesis
or Cleave From Resin
Free Beta-Turn
Mimetic

FIGURE 3